Responses of Rat Basilar Artery to Acetylcholine and Platelet Products In Vivo

Frank M. Faraci, PhD; William G. Mayhan, PhD; and Donald D. Heistad, MD

Studies in vitro suggest that the basilar artery has distinctive responses to endothelium-dependent stimuli. Our first goal was to examine the effects of acetylcholine on diameter of the basilar artery in vivo. Because aggregating platelets may have important effects on cerebral arteries, our second goal was to examine the effects on the basilar artery of products that are released by platelets (thromboxane, serotonin, and adenosine 5'-diphosphate). Diameter of the basilar artery was measured through a cranial window in anesthetized rats (n=25). Baseline diameter of the basilar artery was 247 ±10 μm mean±SEM. Topical application of acetylcholine at 10^-6 and 10^-5 M dilated the basilar artery by 13±2% and 19±2%, respectively. The thromboxane analogue U46619 at 10^-8 and 10^-7 M reduced the diameter of the basilar artery by 18±5% and 29±4%, respectively. At 10^-7 and 10^-6 M, serotonin had little effect on pial arterioles on the cerebrum but constricted the basilar artery by 18±2% and 29±4%, respectively. At 10^-6 and 10^-5 M, adenosine 5'-diphosphate produced marked dilatation of pial arterioles on the cerebrum (9±2% and 20±3%, respectively) but had little effect on the basilar artery (increased diameter by 4±2% and 6±2%, respectively). Thus, in contrast to some studies of the basilar artery in vitro, acetylcholine produces dilatation of the basilar artery in vivo. Potent constrictor responses to thromboxane and serotonin, in combination with the minimal dilator effect of adenosine 5'-diphosphate, suggest that release of these products during platelet aggregation would favor constriction of the basilar artery. (Stroke 1991;22:56-60)
supplemental oxygen. Skeletal muscle paralysis was produced with 5–10 mg·kg⁻¹ gallamine triethiodide. Depth of anesthesia was evaluated by applying pressure to a paw or the tail and observing changes in heart rate or blood pressure. If such changes occurred, additional anesthetic was administered. We have shown previously that this is a sufficient rate of supplementation using nonparalyzed rats. We have also shown that administration of gallamine triethiodide does not alter responses of cerebral arterioles to acetylcholine and other vasoactive stimuli.

A catheter was placed in a femoral artery to measure systemic blood pressure and to obtain arterial blood. A femoral vein was cannulated for infusion of supplemental anesthetic. Arterial blood gases were monitored and maintained within normal limits throughout the experiment (mean ± SEM PaCO₂=37±1 mm Hg, PaO₂=133±8 mm Hg, and pH=7.38±0.01).

A craniotomy was prepared over the ventral brain stem or parietal cortex as described previously in detail. The cranial window was suffused with artificial cerebrospinal fluid (temperature=37–38°C) at 3 ml/min, and a portion of the dura mater was opened. Cerebrospinal fluid sampled from the craniotomies had a mean ± SEM PaCO₂=42±1 mm Hg, PaO₂=57±5 mm Hg, and pH of 7.31±0.02. Diameters of blood vessels were measured using a microscope equipped with a television camera coupled to a video monitor and an image-shearing device. Using this technique, the standard deviation of consecutive measurements is approximately 1% of vessel diameter.

In one group of rats (n=25), we examined responses of the basilar artery to topical suffusion of nitroglycerin (10⁻⁴ to 10⁻⁶ M), acetylcholine (10⁻⁴ to 10⁻⁶ M), the thromboxane A₂ analogue U46619 (10⁻⁹ to 10⁻⁷ M), serotonin (10⁻⁷ to 10⁻⁵ M), and ADP (10⁻⁶ to 10⁻⁴ M). Agonists were mixed in artificial cerebrospinal fluid and suffused over the craniotomy for 5 minutes. Diameter of the basilar artery was measured immediately before and during the last minute of application of each agonist. Following application of a specific agonist, vessels returned to baseline diameter within a few minutes before application of a subsequent agonist. The order of application of agonists was randomized. Application of vehicle (saline or 0.01% ethanol for U46619) did not alter vessel diameter.

For comparison with responses of the basilar artery, we also examined effects of the agonists on pial arterioles on the cerebrum in a second group of rats (n=15). The doses were the same as described for studies of the basilar artery, except that a higher range of doses of serotonin (10⁻⁴ to 10⁻⁶ M) was tested.

Statistical analysis was performed on the absolute values, not the percentage changes, of diameter using repeated-measures analysis of variance; p<0.05 was considered significant. Results are reported as mean±SEM.

**Results**

Under control conditions, diameter of the basilar artery and arteriolar branches of the basilar artery were 247±10 and 88±5 μm, respectively. Pial arterioles on the cerebrum had a diameter of 42±3 μm.

Nitroglycerin produced a dose-related dilatation of the basilar artery and basilar arterioles (Table 1). Blood vessels of the brain stem appeared to be more sensitive than pial arterioles to nitroglycerin. For example, 10⁻⁶ M nitroglycerin in pial arterioles and 10⁻⁸ M nitroglycerin in the basilar artery produced similar degrees of dilatation (15±2% versus 16±1%, respectively) (Table 1).

Acetylcholine at 10⁻⁶ and 10⁻⁵ M produced a dose-dependent dilatation of the basilar artery as well as the basilar and pial arterioles (Table 1). The highest dose of acetylcholine (10⁻⁴ M) tended to produce less dilatation of these vessels.

U46619 produced dose-related constriction of the basilar artery, basilar arterioles, and pial arterioles (Figure 1). Responsiveness to U46619 appeared to be similar for the three groups of vessels.

Serotonin produced dose-related constriction of the basilar artery (Figure 2). The lowest dose of serotonin (10⁻⁹ M) produced significant (p<0.05) vasoconstriction, which indicated that the basilar artery was particularly responsive to this amine. Arteriolar branches of the basilar artery also constricted in response to serotonin in a dose-related manner (Figure 2). In contrast, serotonin had no significant effect on pial arterioles on the cerebrum (Figure 2).

Minimal (6±2% at 10⁻⁵ M) dilatation of the basilar artery was produced by ADP (Figure 3). In contrast, this agent produced marked dose-related dilatation of pial arterioles (Figure 3). While ADP produced some dilatation of basilar arterioles, the response appeared to be less than that observed in pial arterioles.

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<tr>
<th>Table 1. Effects of Nitroglycerin and Acetylcholine on Vessel Diameter in Rats</th>
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<td><strong>Vessel</strong></td>
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<td>Basilar arterioles</td>
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Values are mean±SEM vessel diameter in μm.

*p<0.05 different from control by repeated-measures analysis of variance.

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Discussion

We examined the effects of acetylcholine and the major platelet products on the basilar artery in vivo. There are several major findings. First, in contrast to some studies in vitro, acetylcholine produces dilatation of the basilar artery in vivo. Second, the thromboxane A2 analogue U46619 and serotonin produce constriction of the basilar artery. Pronounced constrictor responses of the basilar artery to serotonin are in striking contrast to the minimal responses of pial arterioles to serotonin. Third, ADP produced minimal dilatation of the basilar artery but marked dilatation of pial arterioles on the cerebrum. Thus, the net effect of vasoactive products that are released by platelets (thromboxane, serotonin, and ADP) favors more constriction in the basilar artery than in pial arterioles.

Several studies have examined the effects of acetylcholine on the basilar artery in vitro. Acetylcholine produces relaxation of the basilar artery from several species including rats.2-4-16-18 In contrast, studies of the effects of acetylcholine on the canine basilar artery in vitro have been inconsistent. Acetylcholine has been reported to produce relaxation,19,20 contraction,6 or no effect.5 It is not clear why the effects of acetylcholine on the canine basilar artery have been inconsistent. Vascular tone is an important determinant of responses in cerebral arteries,10-11 and because isolated vessels have been studied under different levels of tension,5-6,20 it is possible that basal tone contributes to the differences in responses.

Removal of the endothelium abolishes relaxation to acetylcholine of the basilar artery in vitro in several species, including rats.4-16-20 The response to acetylcholine is also attenuated by treatment with hemoglobin.2-17 These findings suggest that relaxation of the basilar artery occurs through an endothelium-dependent mechanism involving the release of an endothelium-derived relaxing factor. In contrast to acetylcholine, nitroglycerin produces relaxation of the basilar artery of rats in vitro through a mechanism that is not dependent on an intact endothelium.15

In pial arterioles in vivo, topical application of acetylcholine produces dilatation and the response is abolished by selective injury to endothelial cells9 or by treatment with methylene blue or hemoglobin, which suggests that acetylcholine reaches the endothelium and produces the local release of endothelium-derived relaxing factor.7-8 In a recent study, inhibition of nitric oxide production with N\(^{G}\)-monomethyl-L-arginine inhibits dilatation of the basilar artery to topical application of acetylcholine in vivo.21

The thromboxane A2 analogue U46619 produces constriction of the basilar artery in vitro.13-22 U46619 also constricts pial arterioles on the cerebrum in several species.15,23,24 Our study indicates that U46619 is a constrictor of the basilar artery in vivo and that responses of the basilar artery are similar to those of pial arterioles.

Effects of serotonin on the cerebral circulation are complex. Serotonin has generally been reported to produce contraction of large cerebral arteries at relatively low doses in vitro.23 In addition, there is
one report of serotonin-induced relaxation of precontracted cerebral arteries in vitro.26 Application of serotonin in vivo has been reported to constrict large pial arteries and to dilate small arterioles in two studies27,28 but to constrict both large and small vessels in another study.29 Serotonin produces constriction of small pial arterioles in mice through an endothelium-dependent mechanism.30 The major finding in our study in relation to serotonin, however, is that in contrast to its minimal effect on pial arterioles, serotonin is a potent constrictor of the basilar artery. In our study, the response of a given group of vessels to serotonin appears to relate to the initial diameter of the vessels. Constrictor responses to serotonin were greater in large vessels than in small vessels. This finding is similar to those of previous studies of pial vessels in vivo.27,28

Endothelium-dependent relaxation of large cerebral arteries and pial arteries in response to ADP has been reported for several species in vitro.31-33 In our study, ADP produced only minimal dilatation of the basilar artery. In contrast, ADP produced marked dilatation of pial arterioles, as observed previously.15 Both nitroglycerin and acetylcholine produced significant dilatation of the basilar artery, which suggests that the modest response to ADP is not related to an impaired dilator capacity of the vessel or to impaired endothelium-dependent relaxation, but seems to be specific for ADP.

We considered the possibility that differences in responses of the basilar artery and pial arterioles to ADP could be related to differences in vessel size. Evidence against this possibility is provided by the finding that constrictor responses to U46619 and dilator responses to nitroglycerin and acetylcholine were similar in the two groups of vessels. Thus, minimal responses of the basilar artery to ADP appear to be specific, and they do not represent a generalized impairment of vasodilator responses.

Our study indicates that the basilar artery dilates in response to acetylcholine in vivo. This response differs from that reported in some studies of the canine basilar artery in vitro but is similar to the classic response of a variety of blood vessels, including pial arterioles, to acetylcholine.

Our results may have implications for responses of the basilar artery to intravascular aggregation of platelets at atherosclerotic lesions or to extravascular aggregation following subarachnoid hemorrhage. The findings that both thromboxane and serotonin produce potent constriction and that ADP produces little dilatation suggest that the net effect of platelets would be constriction of the basilar artery. It is interesting to note that Nihel et al32 recently suggested that vasospasm following subarachnoid hemorrhage tends to occur in large arteries.

References


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